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(FILE 'HCAPLUS' ENTERED AT 08:17:06 ON 22 JUN 2004)

L25 13 S L3-L14 OR L16-L18 OR L20 OR L21 OR L23 OR L24

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L1 5 SEA FILE=REGISTRY LEHDGIN/SQSP  
 L2 5 SEA FILE=REGISTRY L1 AND SQL<=150  
 L3 5 SEA FILE=HCAPLUS L2  
 L4 5 SEA FILE=HCAPLUS FLUOR? AND L3  
 L5 3 SEA FILE=HCAPLUS CASPASE? AND L3  
 L6 0 SEA FILE=HCAPLUS FRET AND L3  
 L7 1 SEA FILE=HCAPLUS FLUORESC?(3A)RESONAN? AND L3  
 L8 1 SEA FILE=HCAPLUS QUENCH? AND L3  
 L9 1 SEA FILE=HCAPLUS L7 AND L8  
 L10 5 SEA FILE=HCAPLUS ?RHODAMIN? AND L3  
 L11 2 SEA FILE=HCAPLUS ?XANTHYLIUM? AND L3  
 L12 4 SEA FILE=HCAPLUS ?COUMARIN? AND L3  
 L13 0 SEA FILE=HCAPLUS ?CARBOCYANIN? AND L3  
 L14 4 SEA FILE=HCAPLUS ?CYANIN? AND L3  
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 L19 93 SEA FILE=HCAPLUS CASPASE? AND ?COUMARIN?  
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 L21 2 SEA FILE=HCAPLUS L19 AND FLUOR? (3A)RESONAN?  
 L22 72 SEA FILE=HCAPLUS CASPASE? AND ?CYANIN?  
 L23 0 SEA FILE=HCAPLUS L22 AND QUENCH?  
 L24 1 SEA FILE=HCAPLUS L22 AND FLUOR? (3A)RESONAN?  
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 L10 OR L11 OR L12 OR L13 OR L14) OR (L16 OR L17 OR L18) OR L20  
 OR L21 OR L23 OR L24

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L25 ANSWER 1 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:414513 HCAPLUS

DOCUMENT NUMBER: 140:419882

ENTRY DATE: Entered STN: 21 May 2004

TITLE: **Fluorescent** peptide substrates for the  
detection of enzyme activity in biological samples

INVENTOR(S): Packard, Beverly S.; Komoriya, Akira

PATENT ASSIGNEE(S): OncoImmunin, Inc., USA

SOURCE: U.S. Pat. Appl. Publ., 114 pp., Cont.-in-part of Appl.  
No. PCT/US00/24882.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

INT. PATENT CLASSIF.:

MAIN: C12Q001-37

SECONDARY: G01N033-573; C07K014-00

US PATENT CLASSIF.: 435023000; 530324000

CLASSIFICATION: 7-1 (Enzymes)

FAMILY ACC. NUM. COUNT: 5

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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US 2004096926      A1  20040520      US 2001-874350      20010604
US 6037137         A   20000314      US 1997-802981      19970220
WO 9837226         A1  19980827      WO 1998-US3000      19980220
W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
    DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG,
    KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX,
    NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
    UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI,
    FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM,
    GA, GN, ML, MR, NE, SN, TD, TG
WO 2001018238      A1  20010315      WO 2000-US24882      20000911
W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
    CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
    HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
    LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
    SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
    YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
    DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
    CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.:      US 1997-802981      A2 19970220
                              WO 1998-US3000      A2 19980220
                              US 1999-394019      A2 19990910
                              WO 2000-US24882      A2 20000911

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## ABSTRACT:

The present invention provides for novel reagents whose **fluorescence** increases in the presence of particular proteases. The reagents comprise a characteristically folded peptide backbone conjugated to two **\*\*\*fluorophores\*\*\*** such that the **fluorophores** are located opposite sides of a cleavage site. When the folded peptide is cleaved, as by digestion with a protease, the **fluorophores** provide a high intensity **\*\*\*fluorescent\*\*\*** signal at a visible wavelength. Because of their high specificity and their high **fluorescence** signal in the visible wavelengths, these protease indicators are particularly well suited for detection of protease activity in biol. samples, in particular in frozen tissue sections. In one example, the protease indicator having the formula F1-Asp-Ala-Ile-Ser-Nle-Pro-Cys-F2, where F1 is a donor **\*\*\*fluorophore\*\*\*** (5-carboxytetramethylrhodamine) linked to aspartic acid via the  $\alpha$ -amino group and F2 is an acceptor **\*\*\*fluorophore\*\*\*** (rhodamine X acetamide (R492)) linked via the sulfhydryl group of the cysteine, exhibits changes in emission spectrum after addn of an elastase protease. Thus this invention also provides for methods of detecting protease activity in situ in frozen sections.

SUPPL. TERM: **fluorescent** peptide substrate enzyme detection;  
 protease detection **fluorescent** peptide substrate;  
 elastase detection **fluorescent** peptide substrate

INDEX TERM: Peptides, uses  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (doubly-labeled; **fluorescent** peptide substrates  
 for the detection of enzyme activity in biol. samples)

INDEX TERM: Cytometry  
 (flow; **fluorescent** peptide substrates for the  
 detection of enzyme activity in biol. samples)

INDEX TERM: Absorption spectroscopy  
 Animal tissue

Blood analysis  
**Cyanine** dyes  
**Fluorescence** microscopy  
**Fluorescent** indicators  
**Fluorometry**  
High throughput screening  
Lymph  
Saliva  
Urine analysis  
    (**fluorescent** peptide substrates for the  
    detection of enzyme activity in biol. samples)  
INDEX TERM: Enzymes, analysis  
ROLE: ANT (Analyte); ANST (Analytical study)  
    (**fluorescent** peptide substrates for the  
    detection of enzyme activity in biol. samples)  
INDEX TERM: Functional groups  
    (hydrophobic; **fluorescent** peptide substrates  
    for the detection of enzyme activity in biol. samples)  
INDEX TERM: 247187-60-6  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
    (apoptosis blocker; **fluorescent** peptide  
    substrates for the detection of enzyme activity in biol.  
    samples)  
INDEX TERM: 171978-35-1  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
    (apoptosis-related protease inhibitor;  
    **fluorescent** peptide substrates for the detection  
    of enzyme activity in biol. samples)  
INDEX TERM: 212268-97-8   212268-98-9   212268-99-0   212269-00-6  
212269-01-7   212269-02-8   212269-03-9   212269-04-0  
212269-05-1   212269-06-2   212269-07-3   212269-08-4  
212269-09-5   212269-10-8  
ROLE: ARG (Analytical reagent use); BSU (Biological study,  
unclassified); ANST (Analytical study); BIOL (Biological  
study); USES (Uses)  
    (cellular uptake; **fluorescent** peptide  
    substrates for the detection of enzyme activity in biol.  
    samples)  
INDEX TERM: 9001-92-7, Proteinase   9004-06-2, Elastase   169592-56-7,  
**Caspase 3**   179241-78-2, **Caspase 8**  
182372-15-2, **Caspase 6**   186322-81-6,  
**Caspase**   189258-14-8, **Caspase 7**  
ROLE: ANT (Analyte); ANST (Analytical study)  
    (**fluorescent** peptide substrates for the  
    detection of enzyme activity in biol. samples)  
INDEX TERM: 596-24-7   989-38-8, Rh6G   2768-89-0, **Rhodamine X**  
20571-42-0   25152-49-2, 9-(2-Carboxyphenyl)-2,7-dimethyl-  
3,6-bis(ethylamino)**xanthylum**   91809-66-4D,  
halides   91809-67-5D, halides   125481-77-8   135926-08-8  
198978-94-8   199608-29-2D, labeled with 6-TMR and/or DER  
203116-52-3   212207-37-9   212207-40-4   220906-39-8  
222164-84-3, Rh 110   690962-76-6D, halides   690962-77-7  
691868-32-3   691868-33-4   691868-34-5   691868-35-6  
ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
USES (Uses)  
    (**fluorescent** peptide substrates for the  
    detection of enzyme activity in biol. samples)

INDEX TERM: 413568-29-3, PhiPhiLux 691873-33-3, CaspaLux-3PE  
 691873-34-4, CaspaLux-6 **691873-35-5**, CaspaLux-9  
 691873-36-6, DCaspaLux-1  
 ROLE: ARG (Analytical reagent use); BSU (Biological study,  
 unclassified); ANST (Analytical study); BIOL (Biological  
 study); USES (Uses)

(**fluorescent** peptide substrates for the  
 detection of enzyme activity in biol. samples)

INDEX TERM: 691510-59-5 691510-60-8 691510-61-9 691510-62-0  
 691510-63-1 691510-64-2 691510-65-3 691510-66-4  
 691510-67-5 691510-68-6 691510-69-7 691510-70-0  
 691510-71-1 691510-72-2 691510-73-3 691510-74-4  
 691510-75-5 691510-76-6 691510-77-7 691510-78-8  
 691510-79-9 691510-80-2 691510-81-3 691510-82-4  
 691510-83-5 691510-84-6 691510-85-7 691510-86-8  
 691510-87-9 691510-88-0 691510-89-1 691510-90-4  
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 691510-95-9 691510-96-0 691510-97-1 691510-98-2  
 691510-99-3 691511-00-9 691511-01-0 691511-02-1  
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 691511-84-9 **691511-85-0** 691511-86-1  
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 691511-99-6 691512-00-2 691512-01-3 691512-02-4  
 691512-03-5 691512-04-6

ROLE: PRP (Properties)

(unclaimed protein sequence; **fluorescent**  
 peptide substrates for the detection of enzyme activity  
 in biol. samples)

INDEX TERM: 637-84-3 150234-52-9 330153-49-6 330153-50-9  
 330574-44-2 330574-45-3 330624-27-6 330624-29-8  
 330624-30-1 330624-31-2 330624-32-3 622400-97-9  
 691388-69-9 691388-71-3 691388-72-4 691388-73-5  
 691388-74-6 691511-48-5

ROLE: PRP (Properties)

(unclaimed sequence; **fluorescent** peptide  
 substrates for the detection of enzyme activity in biol.  
 samples)

L25 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:88323 HCAPLUS

DOCUMENT NUMBER: 140:299199

ENTRY DATE: Entered STN: 04 Feb 2004

TITLE: Fluorescence **Quenching**-Based Assays for Hydrolyzing Enzymes. Application of Time-Resolved Fluorometry in Assays for **Caspase**, Helicase, and PhosphataseAUTHOR(S): Karvinen, Jarkko; Laitala, Ville; Maekinen, Maija-Liisa; Mulari, Outi; Tamminen, Johanna; Hermonen, Jorma; Hurskainen, Pertti; Hemmilae, Ilkka  
CORPORATE SOURCE: PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, FIN-20101, FinlandSOURCE: Analytical Chemistry (2004), 76(5), 1429-1436  
CODEN: ANCHAM; ISSN: 0003-2700

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 7-1 (Enzymes)

ABSTRACT:

We have developed assay technologies to measure hydrolyzing enzymes based on homogeneous time-resolved fluorescence **quenching** (TruPoint). High sensitivity was obtained using fluorescent europium chelates as labels, internally **quenched** by suitable **quenchers** and released upon enzymic reaction. This approach allows robust and sensitive monitoring of low enzyme activities. The assay technol. and the choice of donor-acceptor pairs were evaluated in three different enzymic assays, a protease related to apoptosis, helicase involved in DNA unwinding, and phosphatase having an important role in cellular signaling cascades. All the assays produced an increasing signal, were sensitive, and had a good dynamic range. There were significant differences in optimized **quenchers** for each of the assays depending on the size, flexibility, and rigidity of the substrates. Also, clear differences in the energy-transfer reactions, their requirements for spectral overlapping, ionic interactions, and energy-transfer distances were found. Each of the enzymic assays was briefly tested in a high-throughput screening environment by analyzing signal dynamics and statistical relevance as Z' factors.

SUPPL. TERM: enzyme assay fluorescence **quenching**  
**caspase** DNA helicase CD45 phosphataseINDEX TERM: Enzymes, analysis  
ROLE: ANT (Analyte); ANST (Analytical study)  
(DNA helicase; time-resolved fluorometric assays for **caspase-3**, DNA helicase, and CD45 phosphatase employ Eu3+ chelates in conjunction with chromophore **quenchers**)INDEX TERM: Energy transfer  
(time-resolved fluorometric assays for **caspase-3**, DNA helicase, and CD45 phosphatase employ Eu3+ chelates in conjunction with chromophore **quenchers**)INDEX TERM: Fluorometry  
(time-resolved; time-resolved fluorometric assays for **caspase-3**, DNA helicase, and CD45 phosphatase employ Eu3+ chelates in conjunction with chromophore **quenchers**)INDEX TERM: 169592-56-7, **Caspase-3** 300859-91-0, Protein tyrosine phosphatase CD45  
ROLE: ANT (Analyte); ANST (Analytical study)

(time-resolved fluorometric assays for **caspase**  
-3, DNA helicase, and CD45 phosphatase employ Eu3+  
chelates in conjunction with chromophore  
**quenchers**)

INDEX TERM: 6268-49-1D, Dabcyl, conjugates with peptides and  
oligonucleotides 70281-37-7D, **Tetramethylrhodamine**  
, conjugates with peptides and oligonucleotides  
146368-14-1D, Cy5, conjugates with peptides 247145-23-9D,  
AlexaFluor 546, conjugates with peptides 304014-12-8D, QSY  
7, conjugates with peptides and oligonucleotides  
400051-23-2D, AlexaFluor 647, conjugates with peptides  
477780-06-6D, AlexaFluor 633, conjugates with peptides  
676540-33-3D, Eu-W 1284, conjugates with peptides  
676540-54-8D, Eu-W 8184, conjugates with oligonucleotides  
676540-55-9D, Eu-W 14014, conjugates with peptides  
ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
USES (Uses)

(time-resolved fluorometric assays for **caspase**  
-3, DNA helicase, and CD45 phosphatase employ Eu3+  
chelates in conjunction with chromophore  
**quenchers**)

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS  
RECORD.

REFERENCE(S): (1) Beers, S; Bioorg Med Chem 1997, V5(12), P2201  
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L25 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:892352 HCAPLUS

DOCUMENT NUMBER: 139:379989

ENTRY DATE: Entered STN: 14 Nov 2003

TITLE: Visualization and quantitation of cellular  
cytotoxicity using cell-permeable **fluorogenic**  
protease substrates and **caspase** activity

INVENTOR(S): indicator markers  
 Packard, Beverly; Brown, Martin J.; Feinberg, Mark;  
 Liu, Luzheng; Silvestri, Guido; Chahroudi, Ann;  
 Komoriya, Akira  
 PATENT ASSIGNEE(S): Oncolmmunin, Inc., USA  
 SOURCE: U.S. Pat. Appl. Publ., 25 pp.  
 CODEN: USXXCO  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 INT. PATENT CLASSIF.:  
 MAIN: C12Q001-70  
 SECONDARY: G01N033-53; G01N033-567; C12Q001-37  
 US PATENT CLASSIF.: 435007200; 435023000; 435005000  
 CLASSIFICATION: 15-1 (Immunochemistry)  
 Section cross-reference(s): 9  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003211548	A1	20031113	US 2003-353791	20030128
PRIORITY APPLN. INFO.:			US 2002-353712P P	20020129

## ABSTRACT:

The disclosed invention provides a non-radioactive assay to monitor and quantify the target-cell killing activities mediated by cytotoxic T lymphocytes (CTLs), natural killer cells, and macrophages. This assay is predicated on the discovery that apoptosis pathway activation and, in particular, **caspase** activity, provides a measure of cytotoxic effector cell activity. In one embodiment, measurement of CTL-induced **caspase** activation in target cells is achieved via detection of the specific cleavage of **fluorogenic \*\*\*caspase\*\*\*** substrates. This assay reliably detects antigen-specific CTL killing of target cells, and provides a more sensitive, more informative and safer alternative to the standard 51 Cr-release assay most often used to quantify CTL responses. The assay can be used to study CTL-mediated killing of primary host target cells of different cell lineages, and enables the study of antigen-specific cellular immune responses in real time at the single-cell level. As such, the assay can provide a valuable tool for studies of infectious disease pathogenesis and development of new vaccines and immunotherapies.

SUPPL. TERM: cell cytotoxicity **fluorogenic** protease substrate  
**caspase** marker  
 INDEX TERM: Proteins  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (GDP dissociation inhibitor, G4-GDI; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)  
 INDEX TERM: Ribonucleoproteins  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (HnRNP-C1/2; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)  
 INDEX TERM: Transcription factors  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (SREBP (sterol regulatory element-binding protein);

cellular cytotoxicity determination using cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: Ribonucleoproteins  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (U1 snRNP (U1 snRNA-containing small nuclear ribonucleoprotein); cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Functional groups  
 (aldehyde; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Infection  
 (bacterial; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Labels  
 (calorimetric; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Cytolysis  
 (cell-mediated; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Cerebrospinal fluid  
 Connective tissue  
 Lymph  
 (cell; cellular cytotoxicity determination using

cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: Apoptosis  
 Biomarkers (biological responses)  
 Blood  
 CD8-positive T cell  
 Chromophores  
**Cyanine** dyes  
 Fibroblast  
**Fluorescent** dyes  
**Fluorescent** indicators  
**Fluorescent** substances  
 Immunization  
 Immunotherapy  
 Infection  
 Lymphocytic choriomeningitis virus  
 Macrophage  
 Muscle  
 Neoplasm  
 Osteocyte  
 Post-translational processing  
 Transplant and Transplantation  
 Vaccines  
 (cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Actins  
 Phycoerythrins

ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: T cell (lymphocyte)  
 (cytotoxic, MHC class I-restricted; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: T cell (lymphocyte)  
 (cytotoxic, memory; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: T cell (lymphocyte)  
 (cytotoxic; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Labels  
 (enzymic; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Infection  
 (exposure; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Cytometry  
 (flow; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Labels  
 (**fluorescent**; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Ligands  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (**fluorescent**; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Antibodies and Immunoglobulins  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (**fluorescently** labeled; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Indicators  
 (**fluorogenic**; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Ligands  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
 (**fluorogenic**; cellular cytotoxicity determination using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Organelle

(granule; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Ketones, biological studies  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
(halo, ligands containing; cellular cytotoxicity  
determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Proteins  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
(huntingtin; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Functional groups  
(hydrophobic; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Immunoassay  
(immunofluorescence; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Animal cell  
(inflammatory; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Proteins  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
(lamins, nuclear; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Lymphocyte  
(natural killer cell; cellular cytotoxicity determination  
using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Nerve  
(neuron; cellular cytotoxicity determination using  
cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: Labels  
(radioactive; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Immunization  
(vaccination; cellular cytotoxicity determination using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

INDEX TERM: Infection  
(viral; cellular cytotoxicity determination using  
cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: 1239-45-8, Ethidium bromide 2321-07-5, **Fluorescein**  
2768-89-0, **Rhodamine X** 7240-37-1,

7-Aminoactinomycin D 9001-92-7, Protease 9055-67-8, Poly  
ADP ribose polymerase 13558-31-1, **Rhodamine** 110  
20571-42-0, 7-**Diethylaminocoumarin** 25535-16-4,  
Propidium iodide 70281-37-7, **Tetramethylrhodamine**  
78990-62-2, Calpain 106178-18-1, Granzyme 122191-40-6,  
**Caspase** 1 141436-78-4, Protein kinase C  $\gamma$   
169592-56-7, **Caspase** 3 179241-78-2,  
**Caspase** 8 180189-96-2, **Caspase** 9  
182372-14-1, **Caspase** 2 182372-15-2,  
**Caspase** 6 186322-81-6, **Caspase**  
189303-50-2, Cathepsin W 303752-61-6, DNA protein kinase  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)

(cellular cytotoxicity determination using cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: 158475-79-7 160543-97-5 161928-86-5 202817-40-1  
**330153-12-3** 330153-16-7 612835-70-8  
612835-71-9 612835-72-0 612835-73-1 612835-74-2  
612835-75-3 612835-76-4 612835-77-5 612835-78-6  
612835-81-1

ROLE: PRP (Properties)

(unclaimed protein sequence; visualization and  
quantitation of cellular cytotoxicity using  
cell-permeable **fluorogenic** protease substrates  
and **caspase** activity indicator markers)

L25 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:874871 HCAPLUS

DOCUMENT NUMBER: 139:360902

ENTRY DATE: Entered STN: 07 Nov 2003

TITLE: Homo-doubly **fluorophore**-labeled peptides for  
the detection of enzyme activity in biological samples

INVENTOR(S): Packard, Beverly; Komoriya, Akira

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 42 pp., Cont.-in-part of Appl.  
No. PCT/US00/24882.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

INT. PATENT CLASSIF.:

MAIN: C12Q001-68

SECONDARY: C12Q001-37; C07H021-04; C07K014-47; C12P021-02;  
C12N005-06

US PATENT CLASSIF.: 435006000; 435023000; 435069100; 435320100; 435325000;  
536023100; 530409000; 530410000; 530412000

CLASSIFICATION: 7-1 (Enzymes)

FAMILY ACC. NUM. COUNT: 5

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003207264	A1	20031106	US 2000-747287	20001222
US 6037137	A	20000314	US 1997-802981	19970220
WO 2001018238	A1	20010315	WO 2000-US24882	20000911
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,			

SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,  
 YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,  
 DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,  
 CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  
 WO 2002061038 A2 20020808 WO 2001-US49781 20011221  
 WO 2002061038 C2 20021128  
 WO 2002061038 A3 20030313  
 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT,  
 RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,  
 UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,  
 CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,  
 BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG  
 EP 1356084 A2 20031029 EP 2001-998079 20011221  
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR  
 PRIORITY APPLN. INFO.: US 1997-802981 A2 19970220  
 US 1999-394019 A2 19990910  
 WO 2000-US24882 A2 20000911  
 US 2000-747287 A 20001222  
 WO 2001-US49781 W 20011221

## ABSTRACT:

The present invention provides for novel reagents whose **fluorescence** changes upon cleavage or a change in conformation of a backbone. The reagents comprise a backbone (e.g. nucleic acid, polypeptide, etc.) joining two **fluorophores** of the same species whereby the **fluorophores** form an H-dimer resulting in **quenching** of the **fluorescence** of the **fluorophores**. One such **fluorophore**-labeled peptide comprises DAIP(Nle)SIPKGY, where the **fluorophore** is linked to the N-terminus via the  $\alpha$ -amino group of aspartic acid and to the  $\epsilon$ -amino group of lysine by the displacement of a succinimidyl group linked to 6-**carboxytetramethylrhodamine** (6-TMR) or 5/6-carboxy-X-**rhodamine**. When the backbone is cleaved or changes conformation, the **fluorophores** are separated, no longer forming an H-type dimer, and are de-**quenched** thereby providing a detectable signal. The use of a single **fluorophore** rather than an "acceptor-donor" **fluorescence resonance** energy transfer system offers synthesis and performance advantages. An addnl. discovery of this invention is that attachment of a hydrophobic protecting group to a polypeptide enhances uptake of that polypeptide by a cell. A new class of profluorescent protease substrate was designed and synthesized with spectral properties that fit the exciton model.

SUPPL. TERM: **fluorophore** labeled peptide enzyme detection;  
**fluorescence quenching** labeled peptide  
 enzyme detection; proteinase detection **fluorescence**  
**quenching** labeled peptide  
 INDEX TERM: Antigens  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (detection of interaction with antibody; homo-doubly  
**fluorophore**-labeled peptides for the detection of  
 enzyme activity in biol. samples)  
 INDEX TERM: Antibodies and Immunoglobulins  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (detection of interaction with antigen; homo-doubly  
**fluorophore**-labeled peptides for the detection of

enzyme activity in biol. samples)  
INDEX TERM: Agglutinins and Lectins  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detection of interaction with carbohydrates; homo-doubly  
**fluorophore**-labeled peptides for the detection of  
enzyme activity in biol. samples)  
INDEX TERM: Carbohydrates, analysis  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detection of interaction with lectins; homo-doubly  
**fluorophore**-labeled peptides for the detection of  
enzyme activity in biol. samples)  
INDEX TERM: Receptors  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detection of interaction with ligand; homo-doubly  
**fluorophore**-labeled peptides for the detection of  
enzyme activity in biol. samples)  
INDEX TERM: Nucleic acids  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detection of interaction with nucleic acid-binding  
proteins; homo-doubly **fluorophore**-labeled  
peptides for the detection of enzyme activity in biol.  
samples)  
INDEX TERM: Ligands  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detection of interaction with receptors; homo-doubly  
**fluorophore**-labeled peptides for the detection of  
enzyme activity in biol. samples)  
INDEX TERM: Cytometry  
(flow; homo-doubly **fluorophore**-labeled peptides  
for the detection of enzyme activity in biol. samples)  
INDEX TERM: Absorption spectroscopy  
Animal tissue  
Blood analysis  
Confocal laser scanning microscopy  
Cyanine dyes  
Fluorescence microscopy  
Fluorescence quenching  
Fluorescent substances  
Fluorometry  
Lymph  
Saliva  
Urine analysis  
Yeast  
(homo-doubly **fluorophore**-labeled peptides for  
the detection of enzyme activity in biol. samples)  
INDEX TERM: Enzymes, analysis  
ROLE: ANT (Analyte); ANST (Analytical study)  
(homo-doubly **fluorophore**-labeled peptides for  
the detection of enzyme activity in biol. samples)  
INDEX TERM: Functional groups  
(hydrophobic, cell permeation improvement with;  
homo-doubly **fluorophore**-labeled peptides for  
the detection of enzyme activity in biol. samples)  
INDEX TERM: Animal cell  
(insect; homo-doubly **fluorophore**-labeled  
peptides for the detection of enzyme activity in biol.  
samples)  
INDEX TERM: Animal cell  
(mammalian; homo-doubly **fluorophore**-labeled

peptides for the detection of enzyme activity in biol. samples)

INDEX TERM: Proteins  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (nucleic acid-binding, detection of interaction with nucleic acids; homo-doubly **fluorophore**-labeled peptides for the detection of enzyme activity in biol. samples)

INDEX TERM: 9004-06-2, Elastase 9026-81-7, Nuclease 169592-56-7, CPP32 protease  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (homo-doubly **fluorophore**-labeled peptides for the detection of enzyme activity in biol. samples)

INDEX TERM: 2321-07-5, **Fluorescein** 2768-89-0, **Rhodamine-X** 13558-31-1, **Rhodamine** 110 20571-42-0, 7-**Diethylaminocoumarin** 43070-85-5, **Hydroxycoumarin** 91809-67-5, 6-**Carboxytetramethylrhodamine** 203116-52-3  
 212207-37-9 212207-40-4 212268-88-7 212268-91-2  
 212268-95-6 212268-96-7 618892-30-1 618892-31-2  
 618892-32-3 618892-33-4 618892-34-5 618892-35-6  
 618892-36-7 618892-37-8 618892-38-9 618892-39-0  
 618892-40-3 618892-41-4 618892-42-5 618892-43-6  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (homo-doubly **fluorophore**-labeled peptides for the detection of enzyme activity in biol. samples)

INDEX TERM: 119798-33-3 150234-52-9 150234-53-0 211872-23-0  
 268202-35-3 288310-75-8 330153-28-1 330153-29-2  
 330153-30-5 330153-31-6 330153-32-7 330153-34-9  
 330153-37-2 330153-38-3 330153-39-4 **330153-40-7**  
 330153-41-8 330153-42-9 330153-43-0 330153-44-1  
 330153-45-2 330153-46-3 330153-47-4 330153-48-5  
 330153-50-9 330153-51-0 330624-29-8 330624-30-1  
 330624-31-2 330624-32-3 622400-72-0 622400-73-1  
 622400-74-2 622400-75-3 622400-76-4 622400-77-5  
 622400-78-6 622400-79-7 622400-80-0 622400-81-1  
 622400-82-2 622400-83-3 622400-84-4 622400-85-5  
 622400-86-6 622400-87-7 622400-88-8 622400-89-9  
 622400-90-2 622400-91-3 622400-92-4 622400-93-5  
 622400-94-6 622400-95-7 622400-96-8 622400-97-9  
 622400-98-0 622400-99-1 622401-00-7 622401-01-8  
 622401-02-9 622401-03-0 622401-04-1 622401-05-2  
 622401-06-3 622401-07-4 622401-08-5 622401-09-6  
 622401-10-9 622401-11-0 622401-12-1 622401-13-2  
 622401-14-3 622401-15-4 622401-16-5 622401-17-6  
 622401-18-7 622401-19-8 622401-20-1 622401-21-2  
 622401-22-3 622401-23-4 622401-24-5 622401-25-6  
 622401-26-7 622401-27-8 622401-28-9 622401-29-0  
 ROLE: PRP (Properties)  
 (unclaimed sequence; homo-doubly **fluorophore**-labeled peptides for the detection of enzyme activity in biol. samples)

L25 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN  
 ACCESSION NUMBER: 2003:818208 HCAPLUS  
 DOCUMENT NUMBER: 139:322261  
 ENTRY DATE: Entered STN: 17 Oct 2003  
 TITLE: Visualization and quantitation of cellular

cytotoxicity using cell-permeable **fluorogenic**  
 protease substrates and **caspase** activity  
 indicator markers

INVENTOR(S): Packard, Beverly S.; Brown, Martin J.; Feinberg, Mark;  
 Liu, Luzheng; Silvestri, Guido; Chahroudi, Ann;  
 Komoriya, Akira

PATENT ASSIGNEE(S): Oncoimmunin, Inc., USA

SOURCE: PCT Int. Appl., 61 pp.  
 CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

INT. PATENT CLASSIF.:  
 MAIN: A01N063-00  
 SECONDARY: A61K045-00; C12N005-00; C12Q001-02

CLASSIFICATION: 15-1 (Immunochemistry)  
 Section cross-reference(s): 9

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003084333	A1	20031016	WO 2003-US2583	20030129
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 2002-353112P P 20020129

# ABSTRACT:

This invention provides a non-radioactive assay to monitor and quantify the target-cell killing activities mediated by cytotoxic T lymphocytes (CTLs). This assay is predicated on the discovery that apoptosis pathway activation and, in particular, **caspase** activity, provides a measure of cytotoxic effector cell activity. In one embodiment, measurement of CTL-induced **\*\*\*caspase\*\*\*** activation in target cells is achieved through detection of the specific cleavage of **fluorogenic caspase** substrates. This assay reliably detects antigen-specific CTL killing of target cells, and provides a more sensitive, more informative and safer alternative to the standard <sup>51</sup>Cr-release assay most often used to quantify CTL responses. The assay can be used to study CTL-mediated killing of primary host target cells of different cell lineages, and enables the study of antigen-specific cellular immune responses in real time at the single-cell level. As such, the assay can provide a valuable tool for studies of infectious disease pathogenesis and development of new vaccines and immunotherapies.

SUPPL. TERM: immunocyte cytotoxicity **fluorogenic** substrate  
 protease **caspase**

INDEX TERM: Transcription factors

ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (SREBP (steroid-responsive element-binding protein);  
 visualization and quantitation of cellular cytotoxicity  
 using **caspase** cleavage of)

INDEX TERM: Ribonucleoproteins  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (U1 snRNP (U1 snRNA-containing small nuclear ribonucleoprotein); visualization and quantitation of cellular cytotoxicity using **caspase** cleavage of)

INDEX TERM: Annexins  
 ROLE: ARU (Analytical role, unclassified); ANST (Analytical study)  
 (V, labeled; in visualization and quantitation of cellular cytotoxicity)

INDEX TERM: Animal cell  
 Blood cell  
 Bone  
 Connective tissue  
 Fibroblast  
 Neoplasm  
 (as target cell in visualization and quantitation of cellular cytotoxicity using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Chromophores  
**Fluorescent** substances  
 (**caspase** substrates-containing; in visualization and quantitation of cellular cytotoxicity)

INDEX TERM: T cell (lymphocyte)  
 (cytotoxic, memory; visualization and quantitation of cellular cytotoxicity using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: T cell (lymphocyte)  
 (cytotoxic; visualization and quantitation of cellular cytotoxicity using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Antigens  
 ROLE: ANT (Analyte); BSU (Biological study, unclassified);  
 ANST (Analytical study); BIOL (Biological study)  
 (exoantigens; visualization and quantitation of cellular cytotoxicity using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers in target cells with expression of)

INDEX TERM: Muscle  
 (fiber; as target cell in visualization and quantitation of cellular cytotoxicity using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Cytometry  
 (flow; visualization and quantitation of cellular cytotoxicity using cell-permeable **fluorogenic** protease substrates and **caspase** activity indicator markers)

INDEX TERM: Ribonucleoproteins  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (hnRNP C (heterogeneous nuclear ribonucleoprotein C); visualization and quantitation of cellular cytotoxicity using **caspase** cleavage of)

INDEX TERM: Proteins  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (huntingtin; visualization and quantitation of cellular  
 cytotoxicity using **caspase** cleavage of)

INDEX TERM: Formyl group  
 (in visualization and quantitation of cellular  
 cytotoxicity)

INDEX TERM: Proteins  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (lamins; visualization and quantitation of cellular  
 cytotoxicity using **fluorophore**-labeled  
 monoclonal antibodies to)

INDEX TERM: Antibodies and Immunoglobulins  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (monoclonal, labeled, with **fluorophores**; in  
 visualization and quantitation of cellular cytotoxicity)

INDEX TERM: Lymphocyte  
 (natural killer cell; visualization and quantitation of  
 cellular cytotoxicity using cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: Nerve  
 (neuron; as target cell in visualization and  
 quantitation of cellular cytotoxicity using  
 cell-permeable **fluorogenic** protease substrates  
 and **caspase** activity indicator markers)

INDEX TERM: Cyanine dyes  
 (protease substrates-containing; in visualization and  
 quantitation of cellular cytotoxicity)

INDEX TERM: Phycoerythrins  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (protease substrates-containing; in visualization and  
 quantitation of cellular cytotoxicity)

INDEX TERM: Actins  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (visualization and quantitation of cellular cytotoxicity  
 using **caspase** cleavage of)

INDEX TERM: Apoptosis  
 Cytolysis  
 Cytotoxicity  
**Fluorescence** microscopy  
 Human  
 Macrophage  
 (visualization and quantitation of cellular cytotoxicity  
 using cell-permeable **fluorogenic** protease  
 substrates and **caspase** activity indicator  
 markers)

INDEX TERM: Transplant rejection  
 (visualization and quantitation of cellular cytotoxicity  
 using cell-permeable **fluorogenic** protease  
 substrates and **caspase** activity indicator  
 markers in relation to)

INDEX TERM: Vaccines  
 (visualization and quantitation of cellular cytotoxicity  
 using cell-permeable **fluorogenic** protease

substrates and **caspase** activity indicator  
 markers in relation to screening for antigens for)

INDEX TERM: Animal virus  
 Eubacteria  
 Pathogen  
 (visualization and quantitation of cellular cytotoxicity  
 using cell-permeable **fluorogenic** protease  
 substrates and **caspase** activity indicator  
 markers in target cells infected with)

INDEX TERM: 186322-81-6, **Caspase**  
 ROLE: BSU (Biological study, unclassified); BIOL (Biological  
 study)  
 (**fluorophore**-labeled inhibitors; in  
 visualization and quantitation of cellular cytotoxicity)

INDEX TERM: 2321-07-5D, **Fluorescein**, protease  
 substrates-containing 2768-89-0D, **Rhodamine-X**,  
 protease substrates-containing 13558-31-1D, **Rhodamine**  
 110, protease substrates-containing 20571-42-0D, 7-  
**Diethylaminocoumarin**, protease substrates-containing  
 70281-37-7D, **Tetramethylrhodamine**, protease  
 substrates-containing  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (in visualization and quantitation of cellular  
 cytotoxicity)

INDEX TERM: 1239-45-8, Ethidium bromide 7240-37-1, 7-Amino actinomycin  
 D 25535-16-4, Propidium iodide  
 ROLE: ARU (Analytical role, unclassified); ANST (Analytical  
 study)  
 (in visualization and quantitation of cellular  
 cytotoxicity)

INDEX TERM: 158475-79-7 160543-97-5 161928-86-5 202817-40-1  
 211918-90-0 220846-54-8 **330153-12-3**  
 330153-16-7 612835-70-8 612835-71-9 612835-72-0  
 612835-73-1 612835-74-2 612835-75-3 612835-76-4  
 612835-77-5 612835-78-6 612835-79-7 612835-80-0  
 612835-81-1  
 ROLE: PRP (Properties)  
 (unclaimed sequence; visualization and quantitation of  
 cellular cytotoxicity using cell-permeable  
**fluorogenic** protease substrates and  
**caspase** activity indicator markers)

INDEX TERM: 141436-78-4, Protein kinase Cy 303752-61-6,  
 DNA-dependent protein kinase  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (visualization and quantitation of cellular cytotoxicity  
 using **caspase** cleavage of)

INDEX TERM: 78990-62-2, Calpain 106178-18-1, Granzyme 122191-40-6,  
**Caspase-1** 169592-56-7, **Caspase-3**  
 179241-78-2, **Caspase-8** 180189-96-2,  
**Caspase-9** 182372-14-1, **Caspase-2**  
 182372-15-2, **Caspase-6** 189303-50-2, Cathepsin W  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (visualization and quantitation of cellular cytotoxicity  
 using cell-permeable **fluorogenic** protease  
 substrates and **caspase** activity indicator  
 markers)

INDEX TERM: 9055-67-8, Poly-ADP ribose polymerase

ROLE: ANT (Analyte); ANST (Analytical study)  
(visualization and quantitation of cellular cytotoxicity  
using **fluorophore**-labeled monoclonal antibodies  
to)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS  
RECORD.

REFERENCE(S): (1) Bolton; US 20020058023 A1 2002  
(2) Komoriya; US 6037137 A 2000 HCAPLUS  
(3) Piwnica-Worms; US 6348185 B1 2002 HCAPLUS  
(4) Robinson; US 6395889 B1 2002 HCAPLUS

L25 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:749309 HCAPLUS

DOCUMENT NUMBER: 140:213073

ENTRY DATE: Entered STN: 24 Sep 2003

TITLE: Ca<sup>2+</sup>-dependent and **caspase**-3-independent  
apoptosis caused by damage in Golgi apparatus due to  
2,4,5,7-**tetrabromorhodamine** 123  
bromide-induced photodynamic effects

AUTHOR(S): Ogata, Maiko; Inanami, Osamu; Nakajima, Mihoko;  
Nakajima, Takayuki; Hiraoka, Wakako; Kuwabara,  
Mikinori

CORPORATE SOURCE: Laboratory of Radiation Biology, Department of  
Environmental Veterinary Science, Graduate School of  
Veterinary Medicine, Hokkaido University, Sapporo,  
Japan

SOURCE: Photochemistry and Photobiology (2003), 78(3), 241-247  
CODEN: PHCBAP; ISSN: 0031-8655

PUBLISHER: American Society for Photobiology

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 8-9 (Radiation Biochemistry)

ABSTRACT:

To clarify the role of the Golgi apparatus in photodynamic therapy-induced  
apoptosis, its signaling pathway was studied after photodynamic treatment of  
human cervix carcinoma cell line HeLa, in which a photosensitizer, 2,4,5,7-  
\*\*\***tetrabromorhodamine**\*\*\* 123 bromide (TBR), was incorporated into the Golgi  
apparatus. Laser scanning microscopic anal. of TBR-loaded HeLa cells confirmed that  
TBR was exclusively located in the Golgi apparatus. HeLa cells incubated with TBR  
for 1 h were then exposed to visible light using an Xe lamp. Light of  
wavelength below 670 nm was eliminated with a filter. Morphol. observation of  
nuclei stained with Hoechst 33342 revealed that apoptosis of cells was induced  
by exposure to light. ESR spectrometry showed that light-exposed TBR produced  
both singlet oxygen (<sup>1</sup>O<sub>2</sub>) and superoxide anion (O<sub>2</sub><sup>-</sup>). Apoptosis induction by  
TBR was inhibited by pyrrolidine dithiocarbamate, an O<sub>2</sub><sup>-</sup> scavenger, but not by  
NaN<sub>3</sub>, a **quencher** of <sup>1</sup>O<sub>2</sub>. Furthermore, TBR-induced apoptosis was  
inhibited by aurintricarboxylic acid and ZnCl<sub>2</sub>, which are known as inhibitors  
of DNase (DNase)  $\gamma$ , and (acetoxymethyl)-1,2-bis(o-aminophenoxy)-ethane-  
N,N,N',N'-tetraacetic acid, a chelator of Ca<sup>2+</sup>, but not by acetyl  
Asp-Glu-Val-Asp-aldehyde, an inhibitor of **caspase**-3. These results  
suggested that O<sub>2</sub><sup>-</sup> was responsible for TBR-induced apoptosis, and  
Ca<sup>2+</sup>-dependent and **caspase**-3-independent nuclease such as DNase  
 $\gamma$  played an important role in apoptotic signaling triggered by Golgi  
dysfunction.

SUPPL. TERM: Golgi calcium **caspase** DNase PDT apoptosis

carcinoma

INDEX TERM: Antitumor agents

Apoptosis

Golgi apparatus  
Human  
Oxidative stress, biological  
Photodynamic therapy  
Photosensitizers (pharmaceutical)  
(Golgi apparatus, calcium, **caspase**, and DNase role  
in PDT-induced apoptosis in cervical carcinoma)

INDEX TERM: Uterus, neoplasm  
(cervix, carcinoma; Golgi apparatus, calcium, **caspase**  
, and DNase role in PDT-induced apoptosis in cervical  
carcinoma)

INDEX TERM: 7440-70-2D, Calcium, ions, biological studies 9003-98-9,  
DNase  $\gamma$  169592-56-7, **Caspase 3**  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
(Golgi apparatus, calcium, **caspase**, and DNase role  
in PDT-induced apoptosis in cervical carcinoma)

INDEX TERM: 11062-77-4, Superoxide anion  
ROLE: BSU (Biological study, unclassified); FMU (Formation,  
unclassified); BIOL (Biological study); FORM (Formation,  
nonpreparative)  
(Golgi apparatus, calcium, **caspase**, and DNase role  
in PDT-induced apoptosis in cervical carcinoma)

INDEX TERM: 7782-44-7, Oxygen, biological studies  
ROLE: BSU (Biological study, unclassified); FMU (Formation,  
unclassified); BIOL (Biological study); FORM (Formation,  
nonpreparative)  
(singlet; Golgi apparatus, calcium, **caspase**, and  
DNase role in PDT-induced apoptosis in cervical  
carcinoma)

INDEX TERM: 623903-26-4, **Tetrabromorhodamine 123**  
ROLE: DMA (Drug mechanism of action); PAC (Pharmacological  
activity); PKT (Pharmacokinetics); THU (Therapeutic use);  
BIOL (Biological study); USES (Uses)  
(**tetrabromorhodamine 123**; Golgi apparatus, calcium,  
**caspase**, and DNase role in PDT-induced apoptosis  
in cervical carcinoma)

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS  
RECORD.

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L25 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:241895 HCAPLUS  
 DOCUMENT NUMBER: 138:250716  
 ENTRY DATE: Entered STN: 28 Mar 2003  
 TITLE: Construction of combinatorial libraries of protease  
       fluorogenic substrates and application to substrate  
       profile determination  
 INVENTOR(S): Backes, Bradley J.; Harris, Jennifer Leslie  
 PATENT ASSIGNEE(S): IRM, LLC, Bermuda  
 SOURCE: U.S. Pat. Appl. Publ., 26 pp.  
       CODEN: USXXCO  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 INT. PATENT CLASSIF.:  
       MAIN: G01N033-53  
       SECONDARY: C12Q001-37; C07K007-06; C07K007-08  
 US PATENT CLASSIF.: 435007100; 435023000; 530324000  
 CLASSIFICATION: 7-3 (Enzymes)  
       Section cross-reference(s): 34  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003059847	A1	20030327	US 2002-229950	20020827
WO 2003029823	A1	20030410	WO 2002-US27357	20020827

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,  
 PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,  
 UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD,  
 RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,

CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,  
 PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
 NE, SN, TD, TG

PRIORITY APPLN. INFO.:

US 2001-315116P P 20010827

## ABSTRACT:

Non-peptide protease substrate libraries and high purity protease substrate libraries are constructed using fluorogenic compds. Preparation of the fluorogenic protease substrates is described. The libraries are useful in obtaining substrate profiles for a variety of proteases, such as methods for determining both prime and non-prime protease recognition sequences.

SUPPL. TERM: protease substrate combinatorial library fluorogenic compd  
 INDEX TERM: Peptides, preparation  
 ROLE: MSC (Miscellaneous); SPN (Synthetic preparation); PREP (Preparation)  
 (Fmoc-based peptide synthesis; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Linking agents  
 (ammonia-cleavable, in fluorogenic substrate synthesis; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Acids, reactions  
 Bases, reactions  
 ROLE: RCT (Reactant); RACT (Reactant or reagent)  
 (base-labile protecting group, in fluorogenic substrate synthesis; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Combinatorial library  
 Enzyme kinetics  
 (construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Proteins  
 ROLE: MSC (Miscellaneous)  
 (fluorogenic substrate containing; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Protective groups  
 (in fluorogenic substrate synthesis; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Heterocyclic compounds  
 ROLE: MSC (Miscellaneous)  
 (moiety, protease substrate containing; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Fluorescence  
 Fluorescence excitation  
 Immobilization, molecular or cellular  
 (of fluorogenic compound; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: Solid phase synthesis  
 (of protease substrate; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

**carbamoylmethylcoumarin** 502632-11-3,  
 7-Amino-3-carbomoylmethyl-4-methylcoumarin  
 ROLE: ARG (Analytical reagent use); BSU (Biological study, unclassified); CPS (Chemical process); PEP (Physical, engineering or chemical process); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)  
 (construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: 56-84-8, L-Aspartic acid, reactions  
 ROLE: RCT (Reactant); RACT (Reactant or reagent)  
 (fluorogenic substrate coupling with; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: 100-51-6, Benzylalcohol, reactions 107-21-1, 1,2-Ethanediol, reactions  
 ROLE: NUU (Other use, unclassified); RCT (Reactant); RACT (Reactant or reagent); USES (Uses)  
 (linker, in fluorogenic substrate synthesis; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: 9001-92-7, Protease  
 ROLE: ANT (Analyte); BSU (Biological study, unclassified); CPS (Chemical process); PEP (Physical, engineering or chemical process); ANST (Analytical study); BIOL (Biological study); PROC (Process)  
 (protease; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: 28920-43-6, Fmoc-Cl  
 ROLE: RCT (Reactant); RACT (Reactant or reagent)  
 (protecting group; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: 12794-10-4, Benzodiazepine  
 ROLE: RCT (Reactant); RACT (Reactant or reagent)  
 (solid phase synthesis, fluorogenic substrate preparation; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

INDEX TERM: 9002-88-4, Polyethylene 9003-05-8, Polyacrylamide  
 9003-53-6, Polystyrene 25322-68-3, PEG  
 ROLE: NUU (Other use, unclassified); USES (Uses)  
 (solid support, in fluorogenic substrate synthesis; construction of combinatorial libraries of protease fluorogenic substrates and application to substrate profile determination)

L25 ANSWER 8 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:230990 HCAPLUS

DOCUMENT NUMBER: 139:285536

ENTRY DATE: Entered STN: 25 Mar 2003

TITLE: Single-molecule detection technologies in miniaturized high-throughput screening: Fluorescence intensity distribution analysis

AUTHOR(S): Hapts, Ulrich; Rudiger, Martin; Ashman, Stephen; Turconi, Sandra; Bingham, Ryan; Wharton, Charlotte; Hutchinson, Jonathan; Carey, Charlotte; Moore, Keith J.; Pope, Andrew J.

CORPORATE SOURCE: GlaxoSmithKline, Harlow, UK  
 SOURCE: Journal of Biomolecular Screening (2003), 8(1), 19-33  
 CODEN: JBISF3; ISSN: 1087-0571  
 PUBLISHER: Sage Publications  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 CLASSIFICATION: 1-1 (Pharmacology)

## ABSTRACT:

Single-mol. detection technologies are becoming a powerful readout format to support ultra-high-throughput screening. These methods are based on the anal. of fluorescence intensity fluctuations detected from a small confocal volume element. The fluctuating signal contains information about the mass and brightness of the different species in a mixture. The authors demonstrate a number of applications of fluorescence intensity distribution anal. (FIDA), which discriminates mols. by their specific brightness. Examples for assays based on brightness changes induced by **quenching**/dequenching of fluorescence, fluorescence energy transfer, and multiple-binding stoichiometry are given for important drug targets such as kinases and proteases. FIDA also provides a powerful method to extract correct biol. data in the presence of compound fluorescence.

SUPPL. TERM: miniaturized high throughput screening fluorescence analysis  
 single mol detection; fluorescence intensity distribution  
 analysis miniaturized high throughput drug screening

INDEX TERM: Fluorometry  
 (FIDA (fluorescence intensity distribution anal.);  
 single-mol. detection technol. in miniaturized  
 high-throughput screening using fluorescence intensity  
 distribution anal. (FIDA))

INDEX TERM: RNA  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (binding; single-mol. detection technol. in miniaturized  
 high-throughput screening using fluorescence intensity  
 distribution anal. (FIDA))

INDEX TERM: High throughput screening  
 (drug; single-mol. detection technol. in miniaturized  
 high-throughput screening using fluorescence intensity  
 distribution anal. (FIDA))

INDEX TERM: Drug screening  
 (high throughput; single-mol. detection technol. in  
 miniaturized high-throughput screening using fluorescence  
 intensity distribution anal. (FIDA))

INDEX TERM: Ras proteins  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (p21ras; single-mol. detection technol. in miniaturized  
 high-throughput screening using fluorescence intensity  
 distribution anal. (FIDA))

INDEX TERM: **Fluorescence resonance** energy transfer  
 (single-mol. detection technol. in miniaturized  
 high-throughput screening using fluorescence intensity  
 distribution anal. (FIDA))

INDEX TERM: 9001-84-7, Phospholipase A2 62996-74-1, Staurosporine  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (binding; single-mol. detection technol. in miniaturized  
 high-throughput screening using fluorescence intensity  
 distribution anal. (FIDA))

INDEX TERM: 9001-78-9, Alkaline phosphatase 9025-26-7, Cathepsin D  
 9031-72-5, Alcohol dehydrogenase 67382-96-1,  
 Melanin-concentrating hormone 141349-89-5, p60Src tyrosine

kinase 169592-56-7, **Caspase-3**

ROLE: ANT (Analyte); ANST (Analytical study)

(single-mol. detection technol. in miniaturized  
high-throughput screening using fluorescence intensity  
distribution anal. (FIDA))

INDEX TERM:

58-85-5, Biotin 60-23-1, Mercaptoethylamine 2321-07-5,  
Fluorescein 9013-20-1, Streptavidin 70281-37-7,

**Tetramethylrhodamine** 146368-16-3 189200-71-3,  
**Rhodamine green**

ROLE: ARG (Analytical reagent use); ANST (Analytical study);

USES (Uses)

(single-mol. detection technol. in miniaturized  
high-throughput screening using fluorescence intensity  
distribution anal. (FIDA))

REFERENCE COUNT:

40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS  
RECORD.

REFERENCE(S):

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V1, P3 HCAPLUS
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L25 ANSWER 9 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:578878 HCAPLUS  
 DOCUMENT NUMBER: 138:165551  
 ENTRY DATE: Entered STN: 05 Aug 2002  
 TITLE: A homogeneous **caspase-3** activity assay using  
 HTRF technology  
 AUTHOR(S): Preaudat, M.; Ouled-Diaf, J.; Alpha-Bazin, B.; Mathis,  
 G.; Mitsugi, T.; Aono, Y.; Takahashi, K.; Takemoto, H.  
 CORPORATE SOURCE: Research and New Technologies, CIS Biointernational,  
 Bagnols-sur-Ceze, Fr.  
 SOURCE: Journal of Biomolecular Screening (2002), 7(3),  
 267-274  
 CODEN: JBISF3; ISSN: 1087-0571  
 PUBLISHER: Mary Ann Liebert, Inc.  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 CLASSIFICATION: 7-1 (Enzymes)  
 Section cross-reference(s): 1

ABSTRACT:

**Caspases** are cysteine proteases presenting a conserved active site that cleaves protein substrates at a highly specific position. They are involved in different aspects of the active cell death pathway. Most of them act through proteolytic degrdns. of cellular components. This paper describes the assay development, assay validation, and screening for inhibitors of this enzyme, which could be potential drug candidates. The assay uses homogeneous time-resolved fluorescence based on energy transfer from europium cryptate as donor to cross-linked **allophycocyanin** as acceptor (XL665). A double-tagged substrate, biotinyl-.vepsiln.-aminocaproyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartyl-L-alanylL-propyl-N.vepsiln.-(2,4-dinitrophenyl)-L-lysine-amide (biotin-X-DEVDPK(dnp)-NH<sub>2</sub>), is conjugated with streptavidin cryptate and anti-dnp-XL665 monoclonal antibody. The close proximity between donor and acceptor induces a specific time-resolved fluorescence signal. In the presence of enzyme activity, the substrate cleavage induces an unlinking of the two fluorescent probes and, subsequently, the disappearance of the specific signal as a result of loss of proximity. Expts. to optimize the reagent concentration, incubation times, precision, reproducibility, and robustness are discussed in comparison with a fluorometric method.

SUPPL. TERM: **caspase 3** HTRF detn inhibitor screening  
 INDEX TERM: **Allophycocyanins**  
 ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
 USES (Uses)  
 (cross-linked; homogeneous **caspase-3** activity  
 assay using HTRF technol. and application to inhibitor  
 screening)  
 INDEX TERM: Solubility  
 (effect of solubility of peptide substrate on HTRF assay  
 precision; homogeneous **caspase-3** activity assay  
 using HTRF technol. and application to inhibitor  
 screening)  
 INDEX TERM: Drug screening  
**Fluorescence resonance** energy transfer  
 Fluorescent indicators

INDEX TERM: (homogeneous **caspase-3** activity assay using  
HTRF technol. and application to inhibitor screening)  
Antibodies and Immunoglobulins  
ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
USES (Uses)  
(monoclonal, anti-dnp-XL665, conjugates with biotinylated  
labeled peptide and streptavidin cryptate; homogeneous  
**caspase-3** activity assay using HTRF technol. and  
application to inhibitor screening)

INDEX TERM: 26093-31-2, 7-Amino-4-methyl **coumarin**  
ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
USES (Uses)  
(HTRF assay comparison with; homogeneous **caspase**  
-3 activity assay using HTRF technol. and application to  
inhibitor screening)

INDEX TERM: 184179-08-6  
ROLE: BSU (Biological study, unclassified); BIOL (Biological  
study)  
(**caspase-3** inhibitor; homogeneous  
**caspase-3** activity assay using HTRF technol. and  
application to inhibitor screening)

INDEX TERM: 497178-91-3 497178-92-4 497178-93-5  
ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
USES (Uses)  
(effect of solubility of peptide substrate on HTRF assay  
precision; homogeneous **caspase-3** activity assay  
using HTRF technol. and application to inhibitor  
screening)

INDEX TERM: 169592-56-7, **Caspase-3**  
ROLE: ANT (Analyte); ANST (Analytical study)  
(homogeneous **caspase-3** activity assay using  
HTRF technol. and application to inhibitor screening)

INDEX TERM: 9013-20-1D, Streptavidin, cryptate derivative, conjugates with  
biotinylated labeled peptide and anti-dnp-XL665 monoclonal  
antibody 23978-55-4D, streptavidin derivative, conjugates with  
biotinylated labeled peptide and anti-dnp-XL665 monoclonal  
antibody 128703-72-0 497178-90-2D, conjugates with  
streptavidin cryptate and anti-dnp-XL665 monoclonal antibody  
ROLE: ARG (Analytical reagent use); ANST (Analytical study);  
USES (Uses)  
(homogeneous **caspase-3** activity assay using  
HTRF technol. and application to inhibitor screening)

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS  
RECORD.

REFERENCE(S): (1) Alpha, B; Angew Chem 1987, V26, P266  
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V57, P2197 MEDLINE  
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Conference and Exhibition of the Society for  
Biomolecular Screening 2000  
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- meeting 1994
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Exhibition of the Society for Biomolecular  
Screening 1997
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HCAPLUS
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P279 HCAPLUS

L25 ANSWER 10 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:438549 HCAPLUS  
DOCUMENT NUMBER: 138:21605  
ENTRY DATE: Entered STN: 11 Jun 2002  
TITLE: Mitochondrial permeability transition can be directly  
monitored in living neurons  
AUTHOR(S): Gillesen, T.; Grasshoff, C.; Szinicz, L.  
CORPORATE SOURCE: Max-Planck-Institut of Psychiatry, Munich, 80804,  
Germany  
SOURCE: Biomedicine & Pharmacotherapy (2002), 56(4), 186-193  
CODEN: BIPHEX; ISSN: 0753-3322  
PUBLISHER: Editions Scientifiques et Medicales Elsevier  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
CLASSIFICATION: 9-5 (Biochemical Methods)  
Section cross-reference(s): 13

ABSTRACT:

Mitochondria have been suggested as key players in apoptotic cell death of neurons and many other tissues because the release of proapoptotic mols. from mitochondria is implicated in **caspase** activation. As a potential release mechanism, the occurrence of a large pore opening in the inner membrane (mitochondrial permeability transition pore, PTP) has been proposed, but has not yet been observed directly in neurons. We investigated whether the calcein/Co2+-**quenching** technique introduced by Petronilli et al. [Biofactors 8 (1998) 263], which allows direct observation of PTP opening, can be applied to neurons. Exposure of calcein-loaded neurons to Co2+ ions resulted in the fading of diffuse cytoplasmic calcein fluorescence, with organelle-restricted fluorescent spots remaining. These spots were colocalized with mitochondrially-entrapped **tetramethylrhodamineethylester** (TMRE) fluorescence and corresponded to colocalization of calcein and TMRE fluorescence in digitonin-permeabilized neurons. Importantly, extensive neuronal calcium loading, which is assumed to induce PTP opening, resulted in significant fading of mitochondrial fluorescence, suggesting the occurrence of a permeability transition. This fluorescence decrease could be completely prevented by the PTP blocker cyclosporin A.

SUPPL. TERM: mitochondria permeability transition pore opening neuron  
calcein cobalt assay; PTP transport neuron calcein cobalt  
**tetramethylrhodamineethylester** method  
INDEX TERM: Pore  
(PTP (permeability transition pore); mitochondrial  
permeability transition pore (PTP) opening can be  
directly monitored in living rat neurons using  
calcein/Co2+-**quenching** technique)  
INDEX TERM: Mitochondria  
(mitochondrial permeability transition pore (PTP) opening

can be directly monitored in living rat neurons using calcein/Co2+-**quenching** technique)

INDEX TERM: Nerve  
(neuron; mitochondrial permeability transition pore (PTP) opening can be directly monitored in living rat neurons using calcein/Co2+-**quenching** technique)

INDEX TERM: Biological transport  
(permeation, via PTP; mitochondrial permeability transition pore (PTP) opening can be directly monitored in living rat neurons using calcein/Co2+-**quenching** technique)

INDEX TERM: 1461-15-0, Calcein 7440-48-4, Cobalt, biological studies  
ROLE: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(mitochondrial permeability transition pore (PTP) opening can be directly monitored in living rat neurons using calcein/Co2+-**quenching** technique)

INDEX TERM: 139626-15-6, **Tetramethylrhodamineethyl ester**  
ROLE: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(use in PTP opening assay; mitochondrial permeability transition pore (PTP) opening can be directly monitored in living rat neurons using calcein/Co2+-**quenching** technique)

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD.

REFERENCE(S): (1) Altschuld, R; Am J Physiol 1992, V262, PH1699 HCAPLUS  
(2) Budd, S; Proc Natl Acad Sci 2000, V97, P6161 HCAPLUS  
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(20) Wallach, D; Anal Chem 1963, V35, P1035  
(21) White, R; J Neurosci 1996, V16, P5688 HCAPLUS  
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L25 ANSWER 11 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:251430 HCAPLUS

DOCUMENT NUMBER: 135:32068

ENTRY DATE: Entered STN: 10 Apr 2001

TITLE: Febrile and acute hyperthermia enhance TNF-induced necrosis of murine L929 fibrosarcoma cells via **caspase**-regulated production of reactive

oxygen intermediates  
 AUTHOR(S): Leroux, E.; Auzenne, E.; Weidner, D.; Wu, Z. Y.;  
 Donato, N. J.; Klostergaard, J.  
 CORPORATE SOURCE: Department of Molecular & Cellular Oncology, MD  
 Anderson Cancer Center, The University of Texas,  
 Houston, TX, 77030, USA  
 SOURCE: Journal of Cellular Physiology (2001), 187(2), 256  
 CODEN: JCLLAX; ISSN: 0021-9541  
 PUBLISHER: Wiley-Liss, Inc.  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 CLASSIFICATION: 14-1 (Mammalian Pathological Biochemistry)  
 Section cross-reference(s): 15

## ABSTRACT:

Previous studies have demonstrated the essential role of TNF-induced reactive oxygen intermediates (ROI) in the necrosis of L929 cells. The authors investigated the mol. basis for the interaction of hyperthermia and TNF in these cells. Hyperthermia, both febrile (40.0-40.5°) and acute (41.5-41.8°), strongly potentiated TNF killing, and sensitization was significantly **quenched** by the antioxidant, BHA. The broad-spectrum \*\*\*caspase\*\*\* inhibitor, Z-VAD, has been shown to markedly increase the TNF sensitivity of L929 cells at 37°; the authors observed that hyperthermia would also enhance the sensitivity of L929 cells to TNF + Z-VAD and that BHA could significantly **quench** the response, as well. The basis for hyperthermic potentiation was unlikely thermally-increased sensitivity to ROI, as treatment with hydrogen peroxide for 24 h killed L929 cells essentially equivalently, whether incubated continuously at 37° or at 40.0-40.5°, or for 2 h at 41.5-41.8°. However, febrile and acute hyperthermia markedly increased TNF-induced production of ROI, with or without Z-VAD. Hyperthermia dramatically accelerated the onset of this production, as well as the onset of necrotic death, as determined by oxidation of dihydro-rhodamine and propidium iodide staining, resp., both of which were significantly \*\*\*quenchable\*\*\* with BHA. The authors conclude that hyperthermia potentiates TNF-mediated killing in this cell model primarily by increasing the afferent, and not the efferent, phase of TNF-induced necrosis.

SUPPL. TERM: fever tumor necrosis factor fibrosarcoma **caspase**  
 reactive oxygen; hyperthermia TNF fibrosarcoma necrosis  
**caspase** reactive oxygen  
 INDEX TERM: Sarcoma  
 (fibrosarcoma; hyperthermia enhances TNF-induced necrosis  
 fibrosarcoma cells via **caspase**-regulated production  
 of reactive oxygen intermediates)  
 INDEX TERM: Fever and Hyperthermia  
 Hyperthermia (therapeutic)  
 (hyperthermia enhances TNF-induced necrosis fibrosarcoma  
 cells via **caspase**-regulated production of reactive  
 oxygen intermediates)  
 INDEX TERM: Reactive oxygen species  
 Tumor necrosis factors  
 ROLE: BAC (Biological activity or effector, except adverse);  
 BSU (Biological study, unclassified); BIOL (Biological  
 study)  
 (hyperthermia enhances TNF-induced necrosis fibrosarcoma  
 cells via **caspase**-regulated production of reactive  
 oxygen intermediates)  
 INDEX TERM: 186322-81-6, **Caspase**  
 ROLE: BPR (Biological process); BSU (Biological study,  
 unclassified); BIOL (Biological study); PROC (Process)

(hyperthermia enhances TNF-induced necrosis fibrosarcoma cells via **caspase**-regulated production of reactive oxygen intermediates)

INDEX TERM: 7782-44-7, Oxygen, biological studies

ROLE: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)

(reactive; hyperthermia enhances TNF-induced necrosis fibrosarcoma cells via **caspase**-regulated production of reactive oxygen intermediates)

L25 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:185948 HCAPLUS

DOCUMENT NUMBER: 134:248826

ENTRY DATE: Entered STN: 16 Mar 2001

TITLE: **Fluorogenic** peptides for the detection of protease activity in biological samples and methods of their use

INVENTOR(S): Komoriya, Akira; Packard, Beverly S.

PATENT ASSIGNEE(S): Oncoimmunin, Inc., USA

SOURCE: PCT Int. Appl., 86 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

INT. PATENT CLASSIF.:  
MAIN: C12Q001-37  
SECONDARY: G01N021-00; G01N021-76; A61K038-00

CLASSIFICATION: 7-1 (Enzymes)

FAMILY ACC. NUM. COUNT: 5

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001018238	A1	20010315	WO 2000-US24882	20000911
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
EP 1214445	A1	20020619	EP 2000-961782	20000911
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL			
JP 2003508080	T2	20030304	JP 2001-521773	20000911
US 2003207264	A1	20031106	US 2000-747287	20001222
US 2004096926	A1	20040520	US 2001-874350	20010604
PRIORITY APPLN. INFO.:			US 1999-394019	A 19990910
			US 1997-802981	A2 19970220
			WO 1998-US3000	A2 19980220
			WO 2000-US24882	W 20000911

OTHER SOURCE(S): MARPAT 134:248826

ABSTRACT:

The present invention provides for novel reagents whose **fluorescence** increases in the presence of particular proteases. The reagents comprise a characteristically folded peptide backbone conjugated to two \*\*\*fluorophores\*\*\* such that the **fluorophores** are located opposite

sides of a cleavage site. When the folded peptide is cleaved, as by digestion with a protease, the **fluorophores** provide a high intensity \*\*\*fluorescent\*\*\* signal at a visible wavelength. Because of their high \*\*\*fluorescence\*\*\* signal in the visible wavelengths, these protease indicators are particularly well suited for detection of protease activity in biol. samples, in particular in frozen tissue sections. Thus, this invention also provides for methods of detecting protease activity in situ in frozen sections.

SUPPL. TERM: **fluorogenic** peptide protease detn biol sample; in situ protease detn **fluorogenic** peptide; frozen tissue protease detn **fluorogenic** peptide

INDEX TERM: Cytometry  
(flow; **fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: Absorption spectroscopy  
Animal tissue  
Blood analysis  
Drug delivery systems  
**Fluorometry**  
Lymph  
Saliva  
Urine analysis  
(**fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: Enzymes, analysis  
ROLE: ANT (Analyte); ANST (Analytical study)  
(**fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: Peptides, uses  
ROLE: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(**fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: Animal cell  
(mammalian, delivery of mols. to; **fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: Biological transport  
(uptake; **fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: 9001-92-7, Protease 9004-06-2, Elastase 169592-56-7, CPP32 protease  
ROLE: ANT (Analyte); ANST (Analytical study)  
(**fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

INDEX TERM: 596-24-7D, 9-(2-Carboxyphenyl)-**xanthylum**, peptides containing 2768-89-0D, **Rhodamine X**, peptides containing 20571-42-0D, 7-**Diethylaminocoumarin**, peptides containing 25152-49-2D, 9-(2-Carboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino) **xanthylum**, peptides containing 25794-80-3D, 9-(2-Carboxyphenyl)-3,6-bis(ethylamino) **xanthylum**,

peptides containing 98181-63-6D, peptides containing  
 119798-33-3D, **fluorogenic** peptides containing  
 125481-77-8D, 9-(2,5-Dicarboxyphenyl)-3,6-bis-  
 (dimethylamino)**xanthylum** chloride (5-TMR),  
 peptides containing 135926-08-8D, 9-(2,6-Dicarboxyphenyl)-3,6-  
 bis-(dimethylamino)**xanthylum** chloride (6-TMR),  
 peptides containing 150234-52-9D, **fluorogenic**  
 peptides containing 198978-94-8D, peptides containing  
 203116-52-3 211872-23-0D, **fluorogenic** peptides  
 containing 212207-37-9, 6-TMR-NorFes-6-TMR 212268-88-7  
 212268-91-2 212268-95-6 212268-97-8 212268-98-9  
 212268-99-0 212269-00-6 212269-01-7 212269-02-8  
 212269-03-9 212269-04-0 212269-05-1 212269-06-2  
 212269-07-3 212269-08-4 212269-09-5 212269-10-8  
 288310-75-8D, **fluorogenic** peptides containing  
 330152-87-9, 6-TMR-K-NorFes-6-TMR 330152-88-0D, peptides  
 containing 330152-89-1D, peptides containing 330152-90-4D,  
 peptides containing 330152-91-5D, peptides containing  
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 containing 330152-94-8D, peptides containing 330152-95-9D,  
 peptides containing 330152-96-0D, peptides containing  
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 containing 330152-99-3D, peptides containing 330153-00-9D,  
 peptides containing 330153-01-0D, peptides containing  
 330153-02-1D, peptides containing 330153-03-2D, peptides  
 containing 330153-04-3D, peptides containing 330153-05-4D,  
 peptides containing 330153-06-5D, peptides containing  
 330153-07-6D, peptides containing 330153-08-7D, peptides  
 containing 330153-09-8D, peptides containing 330153-10-1D,  
 peptides containing 330153-11-2D, peptides containing  
**330153-12-3D**, peptides containing 330153-13-4D,  
 peptides containing 330153-14-5D, peptides containing  
 330153-15-6D, peptides containing 330153-16-7D, peptides  
 containing 330153-17-8D, peptides containing 330153-18-9D,  
 peptides containing 330153-19-0D, peptides containing  
 330153-20-3D, peptides containing 330153-21-4D, peptides  
 containing 330153-22-5D, peptides containing 330153-23-6D,  
 peptides containing 330153-24-7D, peptides containing  
 330153-25-8D, peptides containing 330153-26-9D,  
 9-(2,6-Dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)  
**xanthylum**, halides, peptides containing 330153-27-0D,  
**fluorogenic** peptides containing 330153-28-1D,  
**fluorogenic** peptides containing 330153-29-2D,  
**fluorogenic** peptides containing 330153-30-5D,  
**fluorogenic** peptides containing 330153-31-6D,  
**fluorogenic** peptides containing 330153-32-7D,  
**fluorogenic** peptides containing 330153-33-8D,  
**fluorogenic** peptides containing 330153-34-9D,  
**fluorogenic** peptides containing 330153-35-0D,  
**fluorogenic** peptides containing 330153-36-1D,  
**fluorogenic** peptides containing 330153-37-2D,  
**fluorogenic** peptides containing 330153-38-3D,  
**fluorogenic** peptides containing 330153-39-4D,  
**fluorogenic** peptides containing **330153-40-7D**,  
**fluorogenic** peptides containing 330153-41-8D,  
**fluorogenic** peptides containing 330153-42-9D,  
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**fluorogenic** peptides containing 330153-47-4D,  
**fluorogenic** peptides containing 330153-48-5D,  
**fluorogenic** peptides containing 330153-49-6D,  
**fluorogenic** peptides containing 330153-50-9D,  
**fluorogenic** peptides containing 330153-51-0D,  
**fluorogenic** peptides containing 330443-38-4,  
 6-TMR-K-NorFes-DER 330443-39-5D, 9-(2,5 Or  
 2,6-Dicarboxyphenyl)-2,7-dimethyl-3,6-bisamino  
**xanthylum** (Rh110), peptides containing 330443-40-8D,  
 9-(2,5 Or 2,6-Dicarboxyphenyl)-2,7-dimethyl-3-amino-6-hydroxy-  
**xanthylum** (Blue Rh), peptides containing  
 ROLE: ARG (Analytical reagent use); BPR (Biological  
 process); BSU (Biological study, unclassified); ANST  
 (Analytical study); BIOL (Biological study); PROC (Process);  
 USES (Uses)  
 (fluorogenic peptides for the detection of  
 protease activity in biol. samples and methods of their  
 use)

## INDEX TERM:

330574-44-2	330574-45-3	330624-26-5	330624-27-6
330624-28-7	330624-29-8	330624-30-1	330624-31-2
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330659-16-0	330659-17-1	330659-18-2	330659-19-3
330659-20-6	330659-21-7	330659-22-8	330659-23-9
330659-24-0	330659-25-1	330659-26-2	330659-27-3
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330812-34-5	330812-35-6	330812-36-7	330812-37-8
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330812-67-4	330812-76-5	330812-82-3	330812-83-4
330812-84-5	330812-85-6	330812-86-7	330812-87-8
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330813-04-2	330813-06-4	330813-07-5	330813-08-6
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330813-17-7	330813-18-8	330813-19-9	330813-20-2
330813-21-3	330813-23-5	330813-32-6	330813-39-3
330813-40-6	330813-41-7	330813-42-8	330813-43-9
330813-44-0	330813-45-1	330813-46-2	330813-48-4
330813-49-5	330813-51-9	330813-52-0	330813-53-1
330813-57-5	330813-58-6	330813-59-7	330813-60-0
330813-61-1	330813-62-2	330813-63-3	330813-64-4
330813-68-8	330813-69-9	330813-74-6	330813-75-7
330813-84-8	330813-88-2	330813-89-3	330813-90-6
330813-91-7	330813-94-0	330813-95-1	330813-96-2
330813-97-3	330813-98-4	330813-99-5	<b>330814-00-1</b>
330814-01-2	330814-02-3	330814-03-4	330814-04-5
330814-05-6	330814-06-7	330814-07-8	330814-08-9
330814-09-0	330814-10-3	330814-12-5	330814-13-6

330970-84-8 330970-95-1 330974-77-1 330974-80-6  
 330974-94-2 330975-04-7 330975-05-8 330975-14-9  
 330975-19-4 330975-20-7 330975-21-8 330975-23-0  
 330975-24-1 330975-25-2 330975-26-3 330975-27-4  
 330975-28-5 330975-30-9

ROLE: PRP (Properties)

(unclaimed sequence; **fluorogenic** peptides for the detection of protease activity in biol. samples and methods of their use)

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD.

REFERENCE(S): (1) Dykstra; US 5723288 A 1998 HCAPLUS  
 (2) Komoriya; US 6037137 A 2000 HCAPLUS  
 (3) Schade; US 5804395 A 1998 HCAPLUS

L25 ANSWER 13 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:815094 HCAPLUS

DOCUMENT NUMBER: 133:360594

ENTRY DATE: Entered STN: 21 Nov 2000

TITLE: New fluorescent probe possessing fluorescent substances on both ends of peptide substrate for detecting **caspase** activity through **fluorescence resonance** energy transfer

INVENTOR(S): Nagano, Tetsuo; Kikuchi, Kazuya; Minakami, Susumu

PATENT ASSIGNEE(S): Foundation for Scientific Technology Promotion, Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 16 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

INT. PATENT CLASSIF.:

MAIN: C12Q001-37

SECONDARY: C07K001-13; C07K007-06; G01N021-78

CLASSIFICATION: 9-5 (Biochemical Methods)

Section cross-reference(s): 7

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000316598	A2	20001121	JP 1999-134476	19990514
PRIORITY APPLN. INFO.:			JP 1999-134476	19990514

ABSTRACT:

A new fluorescent probe is provided so that the **caspase** activity is detected by observing the time course of the ratio of the relative fluorescence intensity between two fluorescent groups (D and A) bound to the both ends of the peptide substrate possessing the amino acid sequence (e.g., -GDEVDGVK-, -AYVHDAPVK-) to be specifically cleaved by **caspase**. The combination of the two fluorescent groups is chosen so that the fluorescence spectrum of the fluorescent group D (e.g., lucifer yellow, 6-carboxydichlorofluorescein) excited with visible light overlaps the excitation spectrum of the fluorescent group A (e.g., 5-carboxytetramethylrhodamine, 5-carboxy-X-\*\*\*rhodamine\*\*\*), and therefore, the **fluorescence resonance** energy transfer takes place between the two fluorescent groups separated with the amino acid sequence less than 100Å long.

SUPPL. TERM: fluorescent probe **caspase** peptide substrate sequence; **fluorescence resonance** energy transfer imaging **caspase**

INDEX TERM: . **Resonant** energy transfer  
 (fluorescence; new fluorescent probe possessing  
 fluorescent substances on both ends of peptide substrate  
 for detecting **caspase** activity through  
**fluorescence resonance** energy transfer)

INDEX TERM: Imaging  
 (fluorescent; new fluorescent probe possessing  
 fluorescent substances on both ends of peptide substrate  
 for detecting **caspase** activity through  
**fluorescence resonance** energy transfer)

INDEX TERM: Apoptosis  
 Fluorescence excitation  
 Fluorescent probes  
 Fluorescent substances  
 Protein sequences  
 (new fluorescent probe possessing fluorescent substances  
 on both ends of peptide substrate for detecting  
**caspase** activity through **fluorescence**  
**resonance** energy transfer)

INDEX TERM: Peptides, reactions  
 ROLE: PEP (Physical, engineering or chemical process); RCT  
 (Reactant); PROC (Process); RACT (Reactant or reagent)  
 (new fluorescent probe possessing fluorescent substances  
 on both ends of peptide substrate for detecting  
**caspase** activity through **fluorescence**  
**resonance** energy transfer)

INDEX TERM: 169592-56-7, **Caspase-3** 186322-81-6,  
**Caspase**  
 ROLE: BAC (Biological activity or effector, except adverse);  
 BSU (Biological study, unclassified); BIOL (Biological  
 study)  
 (new fluorescent probe possessing fluorescent substances  
 on both ends of peptide substrate for detecting  
**caspase** activity through **fluorescence**  
**resonance** energy transfer)

INDEX TERM: 82446-52-4, Lucifer yellow 91809-66-4 216699-35-3  
 307926-47-2 307926-48-3 307926-49-4  
 ROLE: PEP (Physical, engineering or chemical process); RCT  
 (Reactant); PROC (Process); RACT (Reactant or reagent)  
 (new fluorescent probe possessing fluorescent substances  
 on both ends of peptide substrate for detecting  
**caspase** activity through **fluorescence**  
**resonance** energy transfer)



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Total number of pages: 4

Remarks:

Order of re-scan issued on .....